Titration of reactive amino groups before and after PEGylation suggested that the triple mutant could accept one more PEG strand per subunit than the wild type enzyme. PEGylation increased the circulating life of both the wild type and mutant EPNP enzymes in mice from ~4 hours to >6 days. After a series of intraperitoneal injections at weekly/biweekly intervals, all mice treated with both unmodified EPNPs, and 10 of 16 mice (60%) injected with PEGylated wild type EPNP, developed high levels of anti-EPNP antibody and a marked decline in circulating life. In contrast, only 2/12 mice (17%) treated with the mutant PEG-EPNP developed rapid clearance; low levels of antibody in these mice did not correlate with circulating life. This strategy was thus successful in substantially reducing immunogenicity even though only 1 of the 3 new lysines became modified after treatment with activated PEG.

The baboon and pig uricase subunits each consist of 304 amino acids, 29 of which (i.e. 1 in about 10 residues) are lysines. Initially attempts to introduce 2 Arg-to-Lys substitutions into the cloned cDNA for baboon uricase, and also a substitution of Lys for a Glu codon at position 208, which is known to be a Lys in the human uricase gene, resulted in an expressed mutant baboon protein which had greatly reduced uricase catalytic activity. It was apparent from this experiment that the ability to maintain uricase enzyme activity after arginine to lysine mutation of the mammalian DNA sequence was not predictable.

Subsequently, it was appreciated that amino acid residue 291 in the baboon uricase is lysine, but the corresponding residue in pig is arginine. The ApaI restiction site present in both cDNAs was exploited to construct a chimeric uricase in which the first 225 amino acids are derived from the pig cDNA and the carboxy terminal 79 are derived from the baboon cDNA. The resulting pig-baboon chimeric (PBC) uricase (SEQ ID NO:2) possesses 30 lysines, one more than either "parental" enzyme. An additional feature of the PBC uricase is that its "baboon" portion differs from human uricase at 4 of 79 amino acid residues, whereas pig and human uricase differ at 10 in the same region. A modified version of PBC was subsequently constructed, which maintains the extra lysine at position 291 and otherwise differs from pig uricase only by a substituion of serine for threonine at residue 301 ("pigKS" uricase (SEQ ID NO:4)). In view of the results described in the preceding paragraph wherein several other insertions of lysines

were deleterious to activity, it was unexpected that the PBC and PKS chimeric uricase were fully as active as compared to the unmutated native pig uricase and approximately more than four fold active than unmutated native baboon uricase.

The present invention provides a recombinant pig-baboon chimeric uricase, composed of portions of the pig and baboon liver uricase sequences. One example of such a chimeric uricase contains the first 225 amino acids from the porcine uricase sequence (SEQ ID NO: 7) and the last 79 amino acids from the baboon uricase sequence (SEQ ID NO: 6) (pig-baboon uricase, or PBC uricase; Figure 6 and SEQ ID NO:2). Another example of such a chimeric uricase contains the first 288 amino acids from the porcine sequence (SEQ ID NO: 7) and the last 16 amino acids from the baboon sequence (SEQ ID NO: 6). Since the latter sequence differs from the porcine sequence at only two positions, having a lysine (K) in place of arginine at residue 291 and a serine (S) in place of threonine at residue 301, this mutant is referred to as pig-K-S or PKS uricase.

Vectors (expression and cloning) including the nucleic acid molecules coding the proteins of the present invention are also provided. Preferred vectors include those exemplified herein. One of ordinary skill will appreciate that nucleic acid molecules may be inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the nucleic acid (DNA) may be linked to appropriate transcriptional and translational regulatory nucleotide sequences recognized by the desired host, although such control elements are generally available in expression vectors used and known in the art. The vector may then be introduced into the host cells through standard techniques. Generally, not all of the host cells will be transformed by the vector. It may be necessary, therefore, to select transformed host cells. One such selection method known in the art involves incorporating into the expression vector a DNA sequence, with any necessary control elements, which codes for a selectable marker trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such a selectable trait may be in another vector which is used to co-transform the desired host cells. The vectors can also include an appropriate promoter, such as a prokaryotic promoter capable of expression (transcripton and translation) of the DNA in a bacterial host cell, such as *E. coli*, transformed therewith.

Many expression systems are available and known in the art, including bacterial (for example *E. Coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

Suitable vectors may include a prokaryotic replicon, such as ColE1 *ori*, for propagation in, for example, a prokaryote. Typical prokaryotic vector plasmids are pUC18, pUC19, pUC322 and pBR329 available from Biorad Laboratories (Richmond, CA) and pTcr99A and pKK223-3 available from Pharmacia (Piscataway, NJ). A typical mammalian cell vector plasmid is pSVL available from Pharmacia (Piscataway, NJ). This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells. An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumor virus long terminal repeat to drive expression of the cloned gene. Useful yeast plasmid vectors are pRS403-406 and pRS413-416, and are generally available from Stratagene Cloning Systems (LaJolla, CA). Plasmids pRS403, pRS404, pRS405, and pRS406 are Yeast Integrating plasmids (Yips) and incorporate the yeast selectable markers *HIS3, TRP1, LEU2 and URA3*. Plasmids pRS413-416 are Yeast Centomere plasmids (Ycps).

Moreover, the present invention provides host cells containing these vectors. Preferred host cells include those exemplified and described herein.

The uricase proteins of the present invention may be conjugated via a biologically stable, nontoxic, covalent linkage to a relatively small number of strands of PEG to improve the biological half-life and solubility of the proteins and reduce their immunoreactivity. Such linkages may include urethane (carbamate) linkages, secondary amine linkages, and amide linkages. Various activated PEGs suitable for such conjugation are commercially available from Shearwater Polymers, Huntsville, AL.

The invention also may be used to prepare pharmaceutical compositions of the uricase proteins as conjugates. These conjugates are substantially non-immunogenic and retain at least 70%, preferably 80%, and more preferably at least about 90% or more of the uricolytic activity of the unmodified enzyme. Water-soluble polymers suitable for use in the present invention include linear and branched poly(ethylene